Continental J. Fisheries and Aquatic Science 1: 1-8, 2007

ASPECTS OF MINERAL COMPOSITION OF MALE AND FEMALE *HETEROBRANCHUS BIDORSALIS* ADULTS EXPOSED TO DIFFERENT CONCENTRATIONS OF BONNY- LIGHT CRUDE Oil

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ABSTRACT
Studies were carried out to assess some macro and trace elements of mineral composition of the male and female *Heterobranchus bidorsalis* adults exposed to graded concentrations (1.00-8.00m/L^{-1}) of Bonny-light crude oil (BLCO). The experiment was monitored for 4 days (toxicity) and 42days (recovery) periods. Significant decreases ($P < 0.05$)in the sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), zinc (Zn), iron (Fe), vanadium (Va), lead (Pb) and manganese (Mn) contents of the male *H. bidorsalis* corresponded with the increasing concentrations of BLCO. In contrast, the female fishes recorded significant increases ($P < 0.05$) in the values of the above elements in their tissues as the concentrations of BLCO increased. Furthermore, the values of Na, K, Mg, Ca, P, Zn, Fe, Va, Pb and Mn recorded in the male fishes where generally lower than those of their female counterparts and the control fish. Increased values of these elements were also recorded during the recovery periods (days 14, 28 and 42) of this study in the magnitudes of 15% at day 14, 20% at day 28 and 20% at day 42. This implied that the removal of crude oil stress during this period improved the quantity of these minerals deposited in the fish tissues. The highest percent proportion of Zn and the lowest proportion of Pb recorded in both male and female *H. bidorsalis* adults agreed with the report of other workers for other fish species.

KEYWORDS: *Heterobranchus bidorsalis*, Mineral composition, Bonny-light crude oil, Toxicity, Recovery.

INTRODUCTION
The giant African catfish, *Heterobranchus* species is one of the easiest and the commonest fish raised in ponds with a remarkable fast growth. Its ability to adapt to crowded pond conditions, accept artificial feed and possess high quality flesh have enabled it to gain tremendous popularity (Reed *et al*., 1967; Bard *et al*., 1976; Olatunde, 1983).

The use of fish and invertebrates as bio-indicators of water quality has been advocated by several workers because they produce evidence of relatively stable concentrations compared to water quality analyses that only indicate short term conditions (Ogbeibu and Victor, 1989; Yamazaki *et al*., 1996). Various methods of collecting and integrating data from many specific tests to arrive at a general assessment of the risk posed by chemical pollutions to the aquatic environment have been developed (Cairns and Dickson, 1978; Calaman *et al*., 1979; Oronsaye and Obano, 1998). These protocols for hazard evaluation provide working models for extrapolation of single species data to ecosystem predictions.

Oil spills constitute one of the most important sources of environment problems in Nigerian petroleum industry. The degree of exposure of aquatic organisms to oil is often assessed by measuring their body burden of petroleum-related aromatic compounds (AC_s) because AC_s is potentially harmful to animals (NRC, 1985). Fish and marine animals extensively metabolize most AC_s in their livers and predominantly excrete them in to bile (Vanarasi *et al*., 1989).

Minerals perform a wide variety of structural, biochemical and physiological functions in fish (DeSilva and Anderson, 1995). Six (6) major elements (Fe, Zn, Mn, Ni, I, Mb and Co) have been identified as essential for animal life (Underwood, 1977). Although most of these elements might be required by fish, only 6 dietary minerals have been shown to be required or utilized by salmonids (DeSilva and Anderson, 1995). Most fish species derive their minerals from food or water in which they live. Sea fish therefore contain more minerals than freshwater fish (Laglar *et al.*, 1977). The higher mineral content (calcium) in female Osteichthyes than in males especially during the breeding season has been suggested to be due to increase in protein bound calcium during the breeding period (Urist and Schyeide, 1961).

Detailed proximate analyses are needed to determine the effects of infiltration of crude oil compounds into the tissues of different sexes and age groups of *Heterobranchus bidorsalis*, since this fish commands high market value in Nigeria. Much of the work on the effect of petroleum hydrocarbons on aquatic organisms have been restricted to studies and testing of single compounds (Anderson, 1971) probably due to difficulties in testing complex mixture of compounds associated with crude oil and petroleum fractions. With the incessant oil spills in Nigeria, and the varying level of petroleum hydrocarbons recorded in the body organs of fishes, frogs and snails (Akingbade, 1991), it is imperative that analysis of the quality of fish flesh exposed to different concentrations of crude oil be carried out. This study therefore presents the results of the exposure of male and female adults of *Heterobranchus bidorsalis* to different concentrations of Bonny- light crude oil and its effects on the mineral composition of the fish.

Table1. Gross and Proximate Compositions of the Diet Fed to Male and Female *Heterobranchus bidorsalis* Adults Stocked in Crude Oil Polluted Water

Feed Ingredients	% Composition
Yellow maize	9.29
Soyabean meal	54.84
Fish meal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin mix[1]	0.60
Mineral mix[2]	2.40
Total	100.00
Nutrients	
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.48
Nitrogen-free-extract	36.46
Total	100.00

[1]Vitamin mix provided the following constituents diluted in cellulose (mg/Kg of diet): thiamin, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3000; niacin, 150; menadione-Na-bisulphate, 80; inositol, 400; biotin, 2; vitamin C, 200; alphatocopherol, 200; cholecalciferol, 1000,000 IU/g.
[2]Contained as g/Kg of premix: $FeSO_4.7H_2O$, 5; $MgSO_4.7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; NaCl, 45; Na_2SO_4, 88; $AlCl_3$, 0.15; $CoCl_2.6H_2O$, 0.05; $CuSO_4.5H_2O$, 0.05; $NaSeO_3$, 0.11; $MnSO_4.H_2O$, 0.70; and cellulose, 380.97

MATERIALS AND METHODS

Six hundred (600) fish specimens of two sexes of *Heterobranchus bidorsalis* (Geoffroy St. Hilaire,1809) adults {mean weight ± standard error (SEM), 141.24 ± 0.16} comprising 300 males and 300 females were randomly stocked in 30 aerated- fitted glass aquaria (55 x 30 x 30cm^3) at 20 fish per aquarium. The experiment was designed to have two sets of aquaria in a 4 x 3 arrangement (Completely Randomized Design) to constitute 24 aquaria inundated with 25cm^3 of dechlorinated tap water and contaminated with 5ml each of Bonny-light crude oil (BLCO) at 1.00, 2.00, 4.00 and 8.00ml L^{-1} concentrations. Six aquaria were not contaminated with BLCO and were left as the controls. Mosquito-mesh nets were used to cover the aquaria to prevent fish escape.

Two experimental periods were adopted for the study. The toxicity period lasted for 4 days (96h) while the recovery period which lasted for 42days were monitored at fortnightly (14days) intervals. Fish were also monitored daily during each study period for mortality and survival records. At the end of the toxicity period, the surviving fish and glass aquaria were washed and replenished with dechlorinated tap water. A 38% crude protein diet (Table 1) was fed to fish at 3% body weight per day ($bw.d^{-1}$) during the toxicity period (4 days) and at 5% $bw.d^{-1}$ during the recovery period (42days). Fish were weighed fortnightly during the recovery period with the aid of a top-loading electronic Mettler balance (Model 600 PT) and the diet to be subsequently administered adjusted in accordance with body weight of fish. The infiltration system of aquaria helped in elimination of faeces and other residues.

The mineral compositions of fish were determined at day 4, 14, 28 and 42 of the study period using the method described by Windham (1996); while that of the diet was determined at the beginning of the experiment. The flame photometric method was used to determine the value of sodium (Na) and potassium (K) while ethylene-diamine-tetra-acetic acid (EDTA) titrations were used for those of calcium (Ca) and magnesium (Mg). Complexometric titration method was used for zinc (Zn). For all other minerals tested, the spectrophotometric method of assessment was employed (Windham, 1996) and these were all compared with calibrated series. All the data obtained were analyzed using descriptive statistics and analysis of variance (ANOVA) to indicate statistical significance ($P < 0.05$) (Steel and Torrie, 1990). The Duncan's (1955) Multiple Range Test method was employed to partition the differences.

RESULTS

The macro-element components of the mineral composition of male and female *Heterobranchus bidorsalis* adults exposed to 1.00-8.00 mL^{-1} concentrations of Bonny-light crude oil (BLCO) are shown in Table 2. The male fish exposed to oil pollutant recorded significantly ($P< 0.05$) lower values of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and phosphorus (P) than the control fish (Table 2). Conversely, the control fish recorded significantly ($P < 0.05$) lower values of Na, K, Mg, Ca and P than those of the female fish exposed to the crude oil concentrations. This trend in values of the minerals of the fish relative to the control was shown both as the toxicity (4days) and the recovery (42days) periods of the study.

Whereas the values of Na, K, Mg, Ca and P in male fish decreased significantly with increasing concentrations of BLCO (1.00-8.00mlL^{-1}) (Table 2), those of the male fish increased significantly ($P < 0.05$). Additionally, the recorded values of Na, K, Mg, Ca and P in male *H.bidorsalis* were lower than those of their female counterparts in both experimental periods. Increases in values of the minerals, irrespective of the BLCO concentration to which the fish were exposed, were noticed at days 14, 28 and 42 of the study period (Table 2). These increases were estimated in the magnitudes of 15% at day 14, 20% at days 28 and 42 respectively.

Table 3 shows the trace element compositions of the test fish. As indicated for the macro- elements, the male *H. bidorsalis* adults exposed to the different concentrations of oil pollutant showed significantly ($P < 0.05$) lower values of zinc (Zn), iron (Fe), vanadium (Va), lead (Pb) and manganese (Mn) than their female counterparts (Table 3), as well as the control fish. The values of the trace elements in the female fish were nonetheless significantly high ($P < 0.05$) than those of the control fish both during the toxicity and recovery periods (Table 3).

Similarly, whereas the values of Zn, Fe, Va, Pb and Mn in the male fish decreased significantly ($P < 0.05$) with the increasing concentration of oil exposure of the fish (Table 3), those of their female counterparts increased significantly ($P < 0.05$) as the concentrations of BLCO to which the fishes were exposed increased. The magnitude of increases in the trace element content of both the male and female *H. bidorsalis* during the recovery period were at the rates of 15% at day 14, 20% at day 28 and 20% at day 42. Expectedly, the values of the macro-elements of fish both at the toxicity and the recovery periods of the study (Table 2) were higher than those of trace–elements (Table 3), except for those of Zn which gave outrageously and comparatively higher values.

DISCUSSION

The need to make an assessment of the level of heavy metal contamination in African aquatic environment has been stated by Calamari and Naeve (1994). Consequently, several pollution monitoring programmes which include the Mediterranean Pollution Monitoring Programme (MEDPOL) covering North, West and Central African Marine Pollution and Research Programme (WACAF3) and the Eastern African Marine Pollution and Research Programme (EAF/6) were established. These authors noted that for effective water pollution control and management, there is need for clear understanding of the principles of metal contamination.

The monovalent cations: sodium (Na^+) and potassium (K^+) are primarily involved in ion transport and exchange in fish. An absolute requirement of Na has been demonstrated only in a few plants. Wetzel (1975) stated that Na requirements are particularly high in some species of blue green algae and argued that K and other elements cannot be substituted for Na. The concentration of divalent metal ions: magnesium (Mg^{2+}), iron (Fe^{2+}) and zinc (Zn^{2+}) measures the total hardness of water bodies. Both the total hardness and alkalinity of water are measured in mg $CaCO_3$/litre, since calcium carbonate usually dominates (Fufeyin, 1994).Since most fish species derive their minerals from food and the ambient water environment in which they inhabit (Laglar *et al.*, 1977), the tendency for the crude oil pollution in our study to affect the mineral intake by the fish from the administered feed (Table 1) and from the BLCO contaminated water (1.00-8.00mlL^{-1}) is high.

The inhibition in deposition of macro- elements (Na, K, Mg, Ca and P) (Table 2) in the male *H. bidorsalis* fed with 38% CP diet in an environment replete with oil pollution was observed to be BLCO concentration dependent. The deposition of these elements was, however, enhanced in the female fishes as the BLCO concentrations increased from1.00 to 8.00mlL^{-1}. This implies that the female *H.bidorsalis* adults were not amenable to incorporate these minerals in their body tissues than their male counterparts. This state of affairs was obvious during the 4 days toxicity and 42 days recovery period. The improvement in the values of these elements during the recovery period (Table 2), implies that the removal of the crude oil stress during this period improvedthe quality of these minerals deposited in the fish. This improvement was also more pronounced in the female than in the male fish.

Similarly, the enhancement in the deposition of trace elements (Zn, Fe, Va, Pb and Mn) in the female *H. bidorsalis* as the BLCO concentrations increased (Table 3) contrasted with the reduction in the values of these elements in the males. This situation also implies that more of these elements were deposited in the females than in the males.

Generally, the results obtained from the analysis of macro and trace elements in this study apparently showed the degree of readiness of fish specimens under crude oil stress to maintain life. It has been indicated that minerals perform a wild variety of structural, biochemical and physiological functions in animals (de Silva and Anderson, 1995). Computing the main values of the trace elements in Tables 2 and 3, it was evident for example that zinc (Zn) constituted the highest percent proportion in the female fish during the toxicity (0.72 ± 0.04%) and 14 days into the recovery (0.81 ± 0.05%) periods. Lead (Pb) was recorded with the least values in the same females during the toxicity (0.03 ± 0.01%) and within 14 days recovery (0.03 ± 0.01%) periods. Amongst the macro-elements, the highest percent proportions in the females were computed as follows: P = 0.03 ± 0.04% (4 days toxicity period) and P = 0.42 ± 0.05% (14 days into the recovery period). The lowest percent proportion of the macro-elements also indicated that Na = 0.01 ± 0.03% (14 days toxicity period) and Na = 0.14 ± 0.02% (14 days into the recovery period). In line with the trend in the values of macro and trace elements already indicated, the male fish specimens of this study exhibited lower mean percent values of these elements than their female counterparts. Our present results are consistent with the report of Otuogbai and Epko (2006) who recorded highest percent proportions of zinc (0.45 ± 0.01%) and lowest percent proportions of lead (0.01 ± 0.00%) in the lungfish *Protopterus annectens* under aestivation.

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Received for Publication: 08/05/2007
Accepted for Publication: 11/07/2007

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Table 2. Macro-Elements of the Mineral Composition (%) of *Heterobranchus bidorsalis* Adults Exposed to Graded concentrations of Bony-light Crude Oil for 4 Days (Toxicity) and 42 Days Recovery) Period

Study Period	Duration (Days)	Nutrient	BLCO Concentration ml L^{-1}: Control 0.00 ml L^{-1} M[7]	Control F[8]	1.00 M	1.00 F	2.00 M	2.00 F	4.00 M	4.00 F	8.00 M	8.00 F
Toxicity Period	4	Na[2]	0.09 ± 0.03[a]	0.10 ± 0.01[a]	0.06 ± 0.01[a]	0.11 ± 0.01[a]	0.05 ± 0.02[ab]	0.12 ± 0.01[ab]	0.04 ± 0.01[ac]	0.13 ± 0.01[abc]	0.03 ± 0.01[ed]	0.14 ± 0.01[abc]
		K[3]	0.13 ± 0.01[a]	0.14 ± 0.02[a]	0.13 ±0.02[a]	0.15 ± 0.03[a]	0.11 ±0.04[a]	0.16 ±0.02[ab]	0.01 ± 0.00[c]	0.17 ±0.03[ab]	0.06 ± 0.01[c]	0.18 ± 0.02[ab]
		Mg4	0.09 ± 0.03[a]	0.10 ± 0.02[a]	0.08 ± 0.03[a]	0.11 ± 0.02[a]	0.06 ± 0.03[a]	0.12 ± 0.02[ab]	0.04 ± 0.02[ab]	0.13 ±0.01[ab]	0.02 ± 0.01[abc]	0.14 ± 0.01[abc]
		Ca[5]	0.23 ± 0.04[a]	0.24 ± 0.03[a]	0.22 ± 0.03[s]	0.25 ± 0.03[a]	0.21 ± 0.05[a]	0.27 ±0.01[ab]	0.20 ± 0.04[ab]	0.28 ±0.04[ab]	0.16 ± 0.04[c]	0.30 ± 0.05[d]
		P[6]	0.31 ± 0.03[a]	0.33 ± 0.04[a]	0.29 ± 0.04[a]	0.34 ± 0.04[a]	0.28 ± 0.06[ab]	0.36 ± 0.06[ab]	0.25 ± 0.04[ab]	0.38 ± 0.04[ab]	0.21 ± 0.03[abc]	0.40 ± 0.04[d]
Recovery Period	14	Na	0.10 ± 0.01[a]	0.12 ± 0.01[a]	0.07 ± 0.02[b]	0.13 ± 0.01[c]	0.06 ± 0.02[b]	0.14 ± 0.01[c]	0.05 ± 0.02[b]	0.14 ± 0.01[d]	0.04 ± 0.02[b]	0.15 ± 0.01[d]
		K	0.15 ± 0.02[a]	0.16 ± 0.01[a]	0.15 ± 0.01[a]	0.17 ± 0.01[a]	0.13 ± 0.01[a]	0.18 ± 0.01[a]	0.02 ± 0.01[b]	0.20 ± 0.03[c]	0.07 ± 0.03[b]	0.21 ± 0.01[c]
		Mg	0.10 ± 0.01[a]	0.12 ± 0.01[a]	0.09 ± 0.03[a]	0.13 ± 0.01[a]	0.07 ± 0.03[ab]	0.14 ± 0.01ab	0.05 ± 0.02[ab]	0.15 ± 0.03[ab]	0.02 ± 0.01[ab]	0.16 ± 0.01[abc]
		Ca	0.26 ± 0.01[a]	0.28 ± 0.02[a]	0.25 ± 0.04[a]	0.29 ± 0.01[a]	0.24 ± 0.03[a]	0.31 ± 0.05[ab]	0.23 ± 0.03[ab]	0.32 ± 0.03[ab]	0.18 ± 0.01[c]	0.35 ± 0.02[d]
		P	0.36 ± 0.02[a]	0.38 ± 0.02[a]	0.33 ± 0.04[a]	0.39 ± 0.02[ab]	0.32 ± 0.03[ab]	0.41 ± 0.03[bc]	0.29 ± 0.03[bcd]	0.44 ± 0.02[b]	0.24 ± 0.01[d]	0.46 ± 0.02[b]
	28	Na	0.12 ± 0.01[a]	0.15 ± 0.01[a]	0.08 ± 0.03[ab]	0.15 ± 0.01[a]	0.07 ± 0.03[b]	0.17 ±0.03[a]	0.06 ±0.03[b]	0.15 ± 0.01[a]	0.05 ± 0.03[b]	0.16 ± 0.01[a]
		K	0.18 ± 0.01[a]	0.19 ± 0.01[a]	0.18 ± 0.01[a]	0.21 ± 0.01[a]	0.17 ± 0.01[a]	0.22 ± 0.01[a]	0.03 ± 0.03[b]	0.24 ± 0.03[abc]	0.08 ± 0.03[bc]	0.25 ± 0.03[abc]
		Mg	0.12 ± 0.01[a]	0.14 ± 0.01[a]	0.11 ± 0.01[a]	0.16 ± 0.01[a]	0.08 ± 0.03[b]	0.17 ± 0.01[ab]	0.06 ± 0.02[b]	0.18 ± 0.01[ab]	0.03 ± 0.01[bc]	0.19 ± 0.01[abc]
		Ca	0.32 ± 0.02[a]	0.34 ± 0.02[a]	0.30 ± 0.01[a]	0.35 ± 0.02[a]	0.29 ± 0.04[ab]	0.37 ± 0.02[ab]	0.28 ± 0.04[ab]	0.38 ± 0.02[abc]	0.22 ± 0.02[d]	0.42 ± 0.02[e]
		P	0.43 ± 0.02[a]	0.46 ± 0.02[a]	0.40 ± 0.02[a]	0.47 ± 0.03[ab]	0.36 ± 0.02[c]	0.49 ± 0.03[ab]	0.35 ± 0.03[c]	0.53 ± 0.03[d]	0.29 ± 0.02[e]	0.60 ± 0.03[f]
	42	Na	0.14 ± 0.01[a]	0.17 ± 0.01[a]	0.10 ± 0.01[ab]	0.18 ± 0.01[ab]	0.08 ± 0.02[bc]	0.20 ± 0.01[abc]	0.07 ± 0.02[b]	0.16 ± 0.01[ab]	0.06 ± 0.02[b]	0.17 ± 0.01[abc]
		K	0.22 ± 0.03[a]	0.23 ± 0.03[a]	0.22 ± 0.01[a]	0.24 ± 0.01[b]	0.20 ± 0.01[a]	0.26 ± 0.03[c]	0.04 ± 0.02[b]	0.28 ± 0.01[c]	0.10 ± 0.01[d]	0.30 ± 0.02[c]
		Mg	0.14 ± 0.01[a]	0.18 ± 0.01[a]	0.13 ± 0.01[a]	0.19 ± 0.01[ab]	0.10 ± 0.01[c]	0.20 ± 0.01[bc]	0.07 ± 0.02[d]	0.22 ± 0.01[bc]	0.04 ± 0.01[d]	0.23 ± 0.02[bc]
		Ca	0.38 ± 0.03[a]	0.36 ± 0.03[a]	0.36 ± 0.03[a]	0.42 ± 0.04[b]	0.35 ± 0.02[a]	0.44 ± 0.03[b]	0.34 ± 0.02[a]	0.46 ± 0.02[b]	0.26 ± 0.01[c]	0.51 ± 0.03[d]
		P	0.52 ± 0.03[a]	0.55 ± 0.04[a]	0.48 ± 0.03[b]	0.56 ± 0.04[a]	0.43 ± 0.03[b]	0.59 ± 0.03[ac]	0.45 ± 0.02[b]	0.64 ± 0.03[d]	0.35 ± 0.01[e]	0.72 ± 0.03[f]

[1]Bonny-crude *light oil,*

[2]Sodium, [3]Potassium, [4]Magnesium, [5]Calcium, [6]Phosphorus, [7]Male. [8]Female, Values in the same row followed by the same superscripts are not significantly different (P > 0.05). Values in the same row followed by Different superscripts are significantly different (P < 0.05).

Table 3. Trace Elements of the Mineral composition (%) of *Heterobranchus bidorsalis* Adults Exposed to B\Graded concentration of Bonny-light Crude Oil for 4 Days (Toxicity) and 42 Days (Recovery) Periods

Study period	Duration (Days)	Nutrient	Control 0.00 ml L^{-1}		BLCO Concentration ml L^{-1} 1.00		2.00		4.00		8.00	
			M[7]	F[8]	M	F	M	F	M	F	M	F
Toxicity Period	4	Zn[2]	0.63 ± 0.02[a]	0.66 ± 0.03[a]	0.51 ± 0.03[b]	0.69 ± 0.04[a]	0.04 ± 0.01[c]	0.72 ± 0.04[d]	0.03 ± 0.01[ac]	0.76 ± 0.03[d]	0.02 ± 0.01[c]	0.80 ± 0.04[de]
		Fe[3]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.02 ±0.01[a]	0.05 ± 0.02[a]	0.01 ±0.00[a]	0.06 ±0.02[a]	0.01 ± 0.00[a]	0.08 0.02[ab]	0.01 ± 0.01[c]	0.08 ± 0.01[ab]
		Va[4]	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.04 ± 0.0[a]	0.07 ± 0.02[a]	0.03 ± 0.01[a]	0.08 ± 0.02[ab]	0.02 ± 0.01[a]	0.08 ±0.02[ab]	0.01 ± 0.00[a]	0.09 ± 0.02[ab]
		Pb[5]	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.01 ± 0.00[s]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	0.03 ±0.01[a]	0.01 ± 0.00[a]	0.03 ±0.01[ab]	0.16 ± 0.04[c]	0.30 ± 0.05[d]
		Mn[6]	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.03 ± 0.01[a]	0.06 ± 0.02[a]	0.02 ± 0.01[a]	0.07 ± 0.02[ab]	0.01 ± 0.00[a]	0.07 ± 0.01[ab]	0.01 ± 0.00[a]	0.08 ± 0.01[ab]
Recovery Period	14	Zn	0.64 ± 0.02[a]	0.65 ± 0.03[a]	0.59 ± 0.02[b]	0.79 ± 0.03[d]	0.83 ± 0.04[e]	0.03 ± 0.01[d]	0.87 ± 0.04[e]	0.03 ± 0.01[d]	0.87 ± 0.04[e]	0.03 ± 0.04[f]
		Fe	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.06 ± 0.02[a]	0.01 ± 0.00[a]	0.07 ± 0.02[ab]	0.01 ± 0.00[a]	0.09 ± 0.02[ab]	0.02 ± 0.01[a]	0.09 ± 0.01[ab]
		Va	0.06 ± 0.01[a]	0.05 ± 0.01[a]	0.05 ± 0.02[a]	0.08 ± 0.02[ab]	0.03 ± 0.01[a]	0.09 ± 0.02[ab]	0.02 ± 0.01[a]	0.09 ± 0.03[ab]	0.02 ± 0.01[a]	0.10 ± 0.02[ab]
		Pb	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	0.04 ± 0.01[a]	0.01 ± 0.00[a]	0.02 ± 0.02[a]	0.02 ± 0.01[a]	0.05 ± 0.02[a]
		Mn	0.06 ± 0.01[a]	0.05 ± 0.01[a]	0.04 ± 0.01[a]	0.07 ± 0.02[ab]	0.02 ± 0.00[a]	0.08 ± 0.02[bc]	0.01 ± 0.00[a]	0.08 ± 0.02[ab]	0.02 ± 0.01[d]	0.09 ± 0.03[ab]
	28	Zn	0.62 ± 0.03[a]	0.64 ± 0.04[a]	0.71 ± 0.04[b]	0.95 ± 0.04[c]	0.06 ± 0.02[d]	0.99 ±0.04[c]	0.04 ±0.01[e]	0.04 ± 0.05[f]	0.04 ± 0.01[e]	0.10 ± 0.04[e]
		Fe	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.07 ± 0.02[ab]	0.01 ± 0.00[a]	0.08 ± 0.02[ab]	0.02 ± 0.01[b]	0.11 ± 0.04[ab]	0.03 ± 0.01[a]	0.11 ± 0.05[ab]
		Va	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.06 ± 0.02[a]	0.10 ± 0.00[a]	0.04 ± 0.01[a]	0.11 ± 0.04[ab]	0.03 ± 0.01[a]	0.11 ± 0.04[ab]	0.03 ± 0.01[a]	0.12 ± 0.01[ab]
		Pb	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.05 ± 0.02[a]	0.02 ± 0.01[a]	0.05 ± 0.02[a]	0.03 ± 0.01[d]	0.06 ± 0.01[a]
		Mn	0.04 ± 0.01[a]	0.05 ± 0.02[a]	0.05 ± 0.01[a]	0.08 ± 0.02[a]	0.03 ± 0.01[a]	0.10 ± 0.04[ab]	0.02 ± 0.01[a]	0.09 ± 0.02[ab]	0.03 ± 0.01[a]	0.11 ± 0.02[ab]
	42	Fe	0.03 ± 0.01[a]	0.05 ± 0.02[a]	0.04 ± 0.01[a]	0.08 ± 0.02[ab]	0.02 ± 0.01[a]	0.09 ± 0.02[ab]	0.03 ± 0.01[a]	0.13 ± 0.02[ab]	0.04 ± 0.01[a]	0.13 ± 0.01[ab]
		Pb	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.04 ± 0.01[a]	0.03 ± 0.01[a]	0.06 ± 0.01[a]	0.03 ± 0.01[a]	0.06 ± 0.01[a]	0.04 ± 0.01[a]	0.07 ± 0.01[ab]
		Mn	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.09 ± 0.02[a]	0.04 ± 0.01[a]	0.12 ± 0.01[ab]	0.03 ± 0.01[b]	0.11 ± 0.01[db]	0.04 ± 0.01[a]	0.08 ± 0.02[a]

[1]Bonny-lihgt crude oil, [2]Zinc, [3]Iron, [4]Vanadium, [5]Lead, [6]Mangenese, [7]Female, values in the same row followed by the same superscripts are not significantly different (P > 0.05). Values in the same row followed by different superscripts differ significantly (P < 0.05).